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Increase in Plasma Matrix Metalloproteinase-9 in Acute Stroke Patients With Thrombolysis Failure

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Background and Purpose—Platelet-rich thrombi are resistant to thrombolytics. Matrix metalloproteinases (MMPs) may be involved in platelet aggregation and contribute to thrombolysis failure in stroke patients.

Methods—Plasma samples from 23 stroke patients who had received thrombolytics and from 47 healthy volunteers were examined for MMP-2 and MMP-9 by both enzyme-linked immunosorbent assays and zymography.

Results—The arteries were recanalized in 15 patients but not in the other 8. The MMP-9 plasma level was significantly higher in patients whose arteries were not recanalized.

Conclusions—MMP-9 may be associated with the formation of a thrombolytics-resistant thrombus. (*Stroke*. 2003;34:e48-e50.)

Key Words: metalloproteinases ■ stroke, ischemic ■ thrombolytic therapy

The failure of thrombolytic-induced recanalization in stroke patients is due partly to the resistance of thrombi to lysis by the thrombolytic agents. Although few reports have identified the factors associated with thrombolysis failure, there is evidence suggesting that platelets play a role in thrombolytic resistance.¹

Matrix metalloproteinase (MMP) plays a key role in remodeling the extracellular matrix. In addition, some evidence suggests that MMPs are involved in platelet aggregation^{2,3} and atherosclerotic plaque rupture,⁴ which may lead to the formation of platelet-rich thrombi.

Subjects and Methods

Among 57 stroke patients who had received thrombolytics during a period of 2 years, 23 patients whose arterial recanalization could be evaluated by angiography (21 by conventional angiography and 2 by MR angiography) and whose blood could be obtained before administration of the thrombolytics were prospectively enrolled in this study. This included 7 patients with intravenous tissue-type plasminogen activator (tPA), 5 with intra-arterial urokinase, and 11 with combined intravenous tPA and intra-arterial urokinase. The institutional review board approved this study, and informed consent was obtained. Arterial patency was assessed by the Thrombolysis in Myocardial Infarction (TIMI) grading system, and patients were grouped into nonrecanalization (TIMI 0 or 1) and recanalization (TIMI 2 or 3).

The patients' blood samples were obtained in a heparinized tube when blood was drawn for the initial laboratory workup. Control blood samples were obtained from volunteers >35 years of age at their annual institutional health examinations. Blood samples were examined for 47 volunteers, 23 men and 24 women, with a mean age of 46 years after exclusion of those with a history of hypertension, diabetes, stroke, coronary artery diseases, inflammatory diseases, or

malignancies and those with blood pressure >140/90 mm Hg, fasting blood sugar >7.7 mmol/L (140 mg/dL), or total cholesterol >6.21 mmol/L (240 mg/dL). Blood samples were immediately refrigerated and centrifuged at 3000 rpm for 15 minutes at 4°C, and the plasma was stored at -80°C until analysis.

The MMP-2 and MMP-9 antigen levels were determined with a commercially available enzyme-linked immunosorbent assay kit (Biotrak, Amersham Biosciences) according to the manufacturer's protocol. Gelatin zymography was performed to confirm the enzyme activities.⁵

The SAS system (version 8.2) was used for statistical analysis. Significance was set at $P < 0.05$.

Results

The thrombolysis failed in 8 of the 23 patients. The demographic and clinical characteristics of the groups were similar (the Table). The median delay from stroke onset to the time when blood was taken was 106 minutes (range, 20 to 270 minutes).

The MMP-9 level was significantly different among the 3 groups. It was highest in the nonrecanalization group ($P < 0.0001$ by the Kruskal-Wallis test) (Figure 1). The median value was 311.6 ng/mL (interquartile range [IQR], 173.2 to 372.3) in the nonrecanalization group, 122.6 ng/mL (IQR, 82.4 to 181.0) in the recanalization group, and 54.2 ng/mL (IQR, 28.2 to 98.0) in the control groups. In contrast, the MMP-2 levels were similar among groups ($P = 0.1741$). The most enzyme activities detected by zymography were comparable to those of the latent MMP (Figure 2).

Hemorrhagic transformations occurred in 5 patients (2 symptomatic) who had higher plasma MMP-9 levels.

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Demographic Characteristics of the Patient Groups

	Nonrecanalization (n=8)	Recanalization (n=15)	P
Median age (range), y	71 (58–76)	61 (25–81)	0.16
Sex (men:women)	4:4	8:7	1.0
Risk factors, n			
Hypertension	7	8	0.18
Diabetes mellitus	4	4	0.37
Smoking	1	6	0.35
Previous stroke	5	8	1.0
Cardiac embolic sources	6	7	0.38
Atrial fibrillation	5	5	0.22
LA swirling	1	4	0.62
Aorta plaques	0	4	0.26
Involved arteries, n			0.24
Middle cerebral artery	3	11	
Internal carotid artery	3	2	
Basilar artery	1	2	
Posterior cerebral artery	1	0	
Median time to (range), min			
Blood sampling	113 (20–195)	90 (50–270)	0.93
Angiography	255 (205–300)	260 (80–405)	0.88
Treatment	170 (105–310)	145 (85–405)	0.35
Routes of thrombolysis, n			0.39
IV tPA	4	3	
IA urokinase	1	4	
IV tPA and IA urokinase	3	8	
Median NIHSS (range)	21 (13–33)	17 (8–30)	0.54
HT, n	4	1	0.03

LA indicates left atrial; IV, intravenous; IA, intra-arterial; and NIHSS, National Institutes of Health Stroke Scale. For comparison between groups, the Wilcoxon 2-sample test was used for age, time, and NIHSS; and Fisher's exact test was used for the others.

Discussion

This study demonstrated that the MMP-9 level was higher in the plasma taken within the early hours after the stroke onset. An increase in MMP-9 levels within 12 hours after onset has been reported in cardioembolic stroke.⁶

High pretreatment plasma MMP-9 levels were associated with thrombolytic resistance in this study. Platelet-rich thrombi are resistant to thrombolytics.¹ MMP is suggested to be involved in platelet aggregation. MMP-1, MMP-2, MMP-3, and MMP-9 have all been identified in human platelets.⁷ The release of MMP-2 mediates platelet aggregation.³ MMP-1 acts as a signaling molecule that regulates the thrombotic events by activating the platelets.² Thromboembolic occlusion of the rat middle cerebral artery causes intravascular platelet-leukocyte aggregation in the ischemic area, which coincides with the increase in MMP-9 and might contribute to the time-dependent resistance to fibrinolysis.⁸ In addition to the direct contribution of MMPs in platelet aggregation, they may mediate the process of platelet-rich thrombus formation in atherosclerotic vessels. Disruption of atherosclerotic plaque depends partially on the MMP-9 ac-

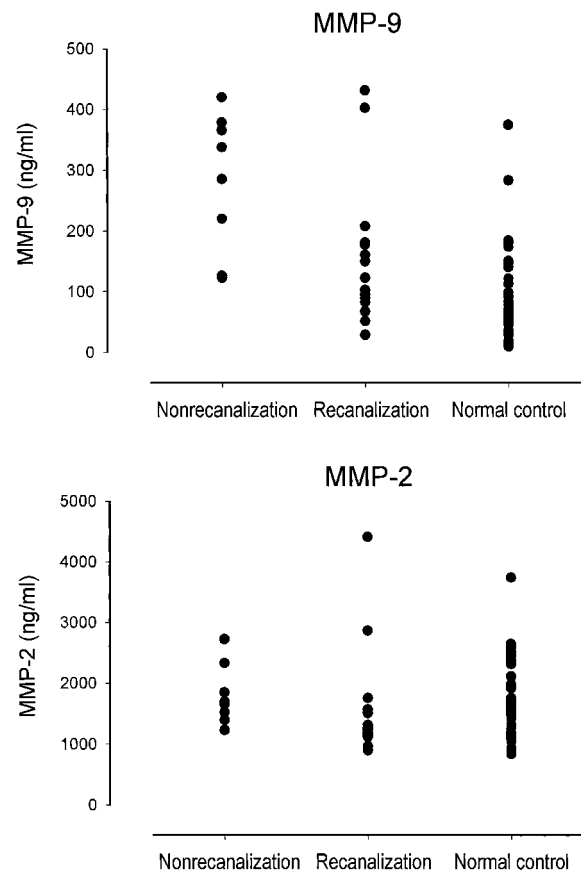


Figure 1. Plasma MMP-2 and MMP-9 levels in each group measured by an enzyme-linked immunosorbent assay.

tivities and amounts.⁴ This indicates that the increases in MMP-9 levels in patients with thrombolysis failure might represent its role in platelet-mediated thrombus formation before and/or after an arterial occlusion, which can result in ineffective thrombolysis.

Hemorrhagic transformations developed more commonly in patients without recanalization. Of note, patients with hemorrhagic transformation had significantly higher baseline plasma MMP-9 levels. The potential role of MMP-9 in hemorrhagic transformation has been demonstrated.⁵ Although MMP-9 might act as a common denominator in both ineffective thrombolysis and hemorrhagic transformation during thrombolytic therapy, the sample size was too small to draw proper conclusions.

This study has several limitations. There were a limited number of patients with different thrombolytic treatment regimens and routes. In addition, the sizes of the occluded arteries were dissimilar. These might account for the lack of statistical significance in certain variables such as the involved arteries and the National Institutes of Health Stroke Scale. Although this study showed for the first time that increased MMP-9 levels are a potential plasma biomarker of thrombolysis failure in strokes, it is unclear whether the increased MMP-9 level contributed to the formation of thrombolytics-resistant thrombi directly or was an epiphenomenon of other factors. Therefore, further studies are necessary to confirm this hypothesis.

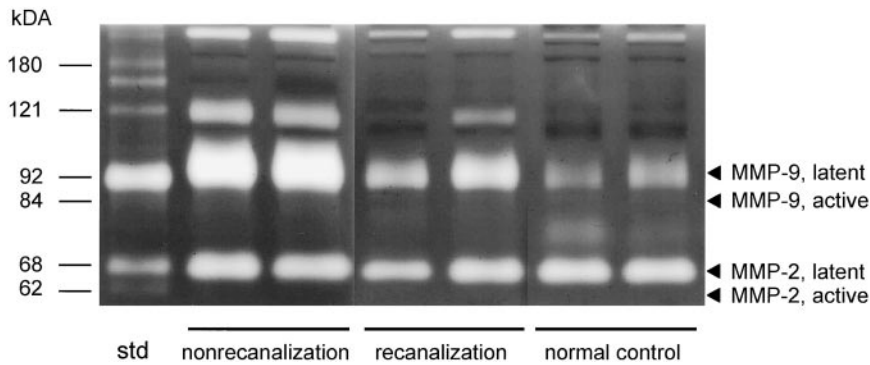


Figure 2. Representative zymogram showing the MMP-2 and MMP-9 bands. Std indicates recombinant MMP standards.

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